



RECEIVED

SEP 29 2003

Exhibit A

TECH CENTER 1600/2810

United States Patent

Famodu, et al.

6,596,926

July 22, 2003

Phosphatidylcholine biosynthetic enzymes

Abstract

This invention relates to an isolated nucleic acid fragment encoding phosphatidylethanolamine N-methyltransferase biosynthetic enzyme. The invention also relates to the construction of a chimeric gene encoding all or a portion of the phosphatidylethanolamine N-methyltransferase biosynthetic enzyme, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of phosphatidylethanolamine N-methyltransferase biosynthetic enzyme in a transformed host cell.

Inventors: **Famodu; Omolayo O.** (Newark, DE); **Kinney; Anthony J.** (Wilmington, DE); **Rafalski; J. Antoni** (Wilmington, DE)

Assignee: **E. I. du Pont de Nemours and Company** (Wilmington, DE)

Appl. No.: **668262**

Filed: **September 22, 2000**

Current U.S. Class: 800/281; 435/6; 435/69.1; 435/183; 435/410; 435/419; 435/252.3; 435/320.1; 530/350; 530/370; 536/23.2; 536/23.6; 536/24.1; 536/24.3; 536/24.33; 800/278; 800/295

Intern'l Class: A01H 003/00; C07H 021/04; C07K 014/415; C12N 005/14; C12N 009/00

Field of Search: 435/6,69.1,183,410,419,252.3,320.1 530/350,370 536/23.2,23.6,24.1,24.3,24.33 800/278,295,281

References Cited [Referenced By]**Other References**

Michael J. Homann et al., *Journal of Bacteriology*, vol. 169(7):3276-3280, Jul. 1987, Coordinate Regulation of Phospholipid Biosynthesis by Serine in *Saccharomyces cerevisiae*.

Xiaoying Lin et al., *Nature*, vol. 402:761-768, Dec. 16, 1999, Sequence and Analysis of Chromosome 2 of the plant *Arabidopsis thaliana*.

Patricia McGraw et al., *Genetics*, vol. 122:317-330, Jun. 1989, Mutations in the *Saccharomyces cerevisiae* *opi3* Gene: Effects on Phospholipid Methylation, Growth and Cross-Pathway Regulation of Inositol Synthesis.

Burgess et al. The Journal of Cell Biology, 1990, vol. 111, p. 2129-2138.*

Brown et al. Science, Nov. 13, 1998, vol. 282, p. 131-133.*

Bork. Genome Research, vol. 10, 2000, p. 398-400.

Primary Examiner: Bui; Phuong T.

Parent Case Text

This application claims the benefit of U.S. Provisional Application No. 60/155,626, filed Sep. 23, 1999.

Claims

What is claimed is:

1. An isolated *polynucleotide* comprising:

(a) a nucleotide sequence encoding a polypeptide having phosphatidylethanolamine N-methyltransferase activity, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:20 have at least 80% sequence *identity* based on the Clustal alignment method, or

(b) the complement of the nucleotide sequence, wherein the complement and the nucleotide sequence contain the same number of nucleotides and are 100% complementary.

2. The *polynucleotide* of claim 1 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:20 have at least 85% sequence *identity* based on the Clustal alignment method.

3. The *polynucleotide* of claim 1 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:20 have at least 90% sequence *identity* based on the Clustal alignment method.

4. The *polynucleotide* of claim 1 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:20 have at least 95% sequence *identity* based on the Clustal alignment method.

5. The *polynucleotide* of claim 1 wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:20.

6. The *polynucleotide* of claim 1 wherein the nucleotide sequence comprises the

USPN 6,596,926

nucleotide sequence of SEQ ID NO:19.

7. A vector comprising the *polynucleotide* of claim 1.

8. A recombinant DNA construct comprising the *polynucleotide* of claim 1 operably linked to a regulatory sequence.

9. A method for transforming a cell comprising transforming a cell with the *polynucleotide* of claim 1.

10. A cell comprising the recombinant DNA construct of claim 8.

11. A method for producing a plant comprising transforming a plant cell with the *polynucleotide* of claim 1 and regenerating a plant from the transformed plant cell.

12. A plant comprising the recombinant DNA construct of claim 8.

13. A seed comprising the recombinant DNA construct of claim 8.

Method for the production of calendic acid, a fatty acid containing delta-8,10,12 conjugated double bonds and related fatty acids having a modification at the delta-9 position

Abstract

The preparation and use of nucleic acid fragments encoding plant fatty acid modifying enzymes associated with modification of the delta-9 position of fatty acids, in particular, formation of conjugated double bonds are disclosed. Chimeric genes incorporating such nucleic acid fragments and suitable regulatory sequences can be used to create transgenic plants having altered lipid profiles. The preparation and use of nucleic acid fragments encoding plant fatty acid modifying enzymes associated with formation of a trans delta-12 double bond also are disclosed. Chimeric genes incorporating such nucleic acid fragments and suitable regulatory sequences can be used to create transgenic plants having altered lipid profiles.

Inventors: **Cahoon; Edgar Benjamin** (Wilmington, DE); **Hitz; William Dean** (Wilmington, DE); **Ripp; Kevin G.** (Wilmington, DE)

Assignee: **E. I. du Pont de Nemours and Company** (Wilmington, DE)

Appl. No.: **638937**

Filed: **August 15, 2000**

Current U.S. Class: 800/281; 800/298; 435/69.1; 435/419; 536/23.6

Intern'l Class: A01H 005/00; C12N 015/82; C07H 021/04

Field of Search: 800/281,298 435/69.1,419 536/23.6

References Cited [Referenced By]

U.S. Patent Documents

<u>4945050</u>	Jul., 1990	Sanford et al.
<u>5107065</u>	Apr., 1992	Shewmaker et al.
<u>5231020</u>	Jul., 1993	Jorgensen et al.
<u>5428072</u>	Jun., 1995	Cook et al.
<u>5519451</u>	May., 1996	Clatanoff et al.
<u>5554646</u>	Sep., 1996	Cook et al.
<u>5851572</u>	Dec., 1998	Cook et al.

Reaction with Single-Sided Specificity: Analysis of T C Receptor 8 Chain.
Nancy H. Wallace et al., Plant Phys., vol. 95:973-975, 1991, Nucleotide
Sequence of a cDNA Clone Corresponding to the Maize Globulin-2 Gene.
Joan T. Odell et al., Nature, vol. 313:810-812, 1985, Identification of DNA
sequences required for activity of the cauliflower mosaic virus 35S Promoter.
Mark D. Adams et al., Science, vol. 252:1651-1656, 1991, Complementary
DNA Sequencing: Expressed Sequence Tags and Human Genome Project.
M. D. Chisholm et al., Can. Journ. of Biochem., vol. 42:1033-1040, 1964,
Biosynthesis of Mustard Oil Glucosides.
R. C. Thiel et al., J. Anim. Sci., vol. 77(suppl):47, 1998, Effects of CLA
supplementation on quality and sensory characteristics of pork.
R. C. Wiegand et al., J. Anim. Sci., vol. 77(suppl):47, 1999, Effects of CLA
supplementation on pork quality characteristics in crossbred growing-finishing
barrows.
Linda Gritz et al., Gene, vol. 25: 179-188, 1983, Plasmid-encoded hygromycin
B resistance: the sequence of hygromycin B phosphotransferase gene and its
expression in Escherichia coli and Saccharomyces cerevisiae.
Mats Hamberg et al., Biochem. & Biophys. Res. Comm., vol. 188(3):1992,
Metabolism of 6,9,12-Octadecatrienoic acid in the red alga lithothamnion
corallioides: Mechanism of formation of a conjugated tetraene fatty acid.
Edgar B. Cahoon et al., J. Biol. Chem., vol. Manuscript Moo9188200, No. in
press, 2000, pp. 1-27, Formation of conjugated delta 8, delta 10 double bonds by
delta 12-oleic acid desaturase related enzymes: Biosynthetic origin of calendic
acid.
Kathrin Fritsche et al., FEBS Lett., vol. 462:249-253, 1999, Isolation and
characterization of a calendic acid producing (8,11)-linoleoyl desaturase.

Primary Examiner: McElwain; Elizabeth F.

Parent Case Text

This application claims priority benefit of U.S. Provisional Application No. 60/149,050
filed Aug. 16, 1999, now abandoned.

Claims

What is claimed is:

1. A chimeric gene comprising an isolated *nucleic acid* fragment encoding a plant fatty
acid modifying enzyme associated with conjugated double bond formation comprising a
delta-9 position of fatty acids having an amino acid *identity* of at least 72.5% based on
the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:2 or 4

wherein said fragment or a functionally equivalent subfragment thereof or a complement thereof is operably linked to suitable regulatory sequences.

2. The chimeric gene of claim 1 wherein the *nucleic acid* fragment is isolated from *Calendula officinalis*.
3. The chimeric gene of claim 1 wherein the plant fatty acid modifying enzyme is associated with the formation of calendic acid.
4. A transformed host cell or plant comprising in its genome the chimeric gene of claim 1.
5. A transformed host cell or plant comprising in its genome the chimeric gene of claim 2.
6. A transformed host cell or plant comprising in its genome the chimeric gene of claim 3.
7. A method of altering the level of fatty acids in a host cell or plant wherein said fatty acids comprise a modification at a delta-9 position, said method comprising:
 - (a) transforming a host cell or plant with the chimeric gene of claim 1;
 - (b) growing the transformed host cell or plant under conditions suitable for the expression of the chimeric gene; and
 - (c) selecting those transformed host cells or plants having altered levels of fatty acids comprising a modified delta-9 position.
8. A method of altering the level of fatty acids in a host cell or plant wherein said fatty acids comprise a modification at a delta-9 position, said method comprising:
 - (a) transforming a host cell or plant with the chimeric gene of claim 2;
 - (b) growing the transformed host cell or plant under conditions suitable for the expression of the chimeric gene; and
 - (c) selecting those transformed host cells or plants having altered levels of fatty acids comprising a modified delta-9 position.
9. A method of altering the level of fatty acids in a host cell or plant wherein said fatty acids comprise a modification at a delta-9 position, said method comprising:
 - (a) transforming a host cell or plant with the chimeric gene of claim 3;
 - (b) growing the transformed host cell or plant under conditions suitable for the expression

of the chimeric gene; and

(c) selecting those transformed host cells or plants having altered levels of fatty acids comprising a modified delta-9 position.

10. The method of claim 7, 8, or 9 wherein the host cell or plant is selected from the group consisting of plant cells and microorganisms.

11. The method of claim 7, 8, or 9 and wherein the level of calendic acid is altered.

12. A method for producing fatty acid modifying enzymes associated with modification of a delta-9 position of fatty acids which comprises:

(a) transforming a microbial host cell with the chimeric gene of claim 1;

(b) growing the transformed host cell under conditions suitable for the expression of the chimeric gene; and

(c) selecting those transformed host cells containing altered levels of protein encoded by the chimeric gene.

13. A method for producing fatty acid modifying enzymes associated with modification of a delta-9 position of fatty acids which comprises:

(a) transforming a microbial host cell with the chimeric gene of claim 2;

(b) growing the transformed host cell under conditions suitable for the expression of the chimeric gene; and

(c) selecting those transformed host cells containing altered levels of protein encoded by the chimeric gene.

14. A method for producing fatty acid modifying enzymes associated with modification of a delta-9 position of fatty acids which comprises:

(a) transforming a microbial host cell with the chimeric gene of claim 3;

(b) growing the transformed host cell under conditions suitable for the expression of the chimeric gene; and

(c) selecting those transformed host cells containing altered levels of protein encoded by the chimeric gene.

15. The method of claim 12, 13, or 14 wherein the fatty acid modifying enzyme is associated with the formation of calendic acid or dimorphecolic acid.

United States Patent
Frohberg

6,590,141
July 8, 2003

Nucleic acid molecules from plants encoding enzymes which participate in starch synthesis

Abstract

Nucleic acid molecules are described which encode enzymes which participate in starch synthesis in plants. These enzymes are a new isoform of starch synthase. There are furthermore described vectors for generating transgenic plant cells and plants which synthesize a modified starch. There are furthermore described methods for the generation of these transgenic plant cells and plants, and methods for producing modified starches.

Inventors: **Frohberg; Claus** (Berlin, DE)

Assignee: **Aventis CropScience GmbH** (Frankfurt, DE)

Appl. No.: **638524**

Filed: **August 11, 2000**

Foreign Application Priority Data

Aug 11, 1999[DE]

199 37 348

Current U.S. Class: 800/284; 800/278; 800/286; 800/320.1; 435/69.1;
435/101; 435/320.1; 435/419; 435/468; 536/23.6

Intern'l Class: C12N 015/29; C12N 015/82; C12N 005/04; A01H
005/00; C12P 019/04

Field of Search: 536/23.6 435/69.1,468,320.1,419,101
800/278,284,320.1,286

References Cited [Referenced By]

U.S. Patent Documents

6130367 Oct., 2000 Kossmann et al.

Foreign Patent Documents

2242398 Jul., 1997 CA.

2255538 Nov., 1997 CA.

196 19 918 Nov., 1997 DE.

196 53 176 Jun., 1998 DE.

0 779 363 Jun., 1997 EP.

USPN 6,590,141

WO 96/15248	May., 1996	WO.
WO 97/263362	Jul., 1997	WO.
WO 98/27212	Jun., 1998	WO.
WO 99/24575	May., 1999	WO.

Other References

Nakatani et al. Jpn. J. Crop Sci. 61(3): 463-468, 1992.*

Kossmann et al. Progress Biotechnol. 10:271-278, 1995.*

Sasaki, T. Accession No. C99345, 1996.*

Database Accession No. C99345 published Oct. 16, 1998, also referred to as XP 002163786.

Database Accession No. AI94842 published Aug. 23, 1999, also referred to as XP 002163787.

Cao, Heping et al, "Identification of the Soluble Starch Synthase Activities of Maize Endosperm", Plant Physiology, May 1999, vol. 120, pp. 205-215, No. 1.

Primary Examiner: Fox; David T.

Attorney, Agent or Firm: Frommer Lawrence & Haug LLP

Claims

I claim:

1. An isolated **nucleic acid** molecule encoding a protein with the bioactivity of a starch synthase selected from the group consisting of

(a) **nucleic acid** molecules which encode a protein with the amino acid sequence indicated under SEQ ID No. 2;

(b) **nucleic acid** molecules which encompass the nucleotide sequence shown under SEQ ID No. 1 or a complementary sequence thereof;

(c) **nucleic acid** molecules which encompass the coding region of the nucleotide sequence of the cDNA present in plasmid IR 65/87 (deposit number DSM 12970) or a complementary sequence thereof;

(d) **nucleic acid** molecules whose nucleotide sequence deviates from the sequence of the **nucleic acid** molecules mentioned under (a), (b) or (c) owing to the degeneracy of the genetic code;

(e) **nucleic acid** molecules which have over 85% sequence *identity* with SEQ ID NO:1;
and

(f) **nucleic acid** molecules which constitute allelic variants of the nucleic acid molecules indicated under (a), (b), (c), (d) or (e).

2. The **nucleic acid** molecule as claimed in claim 1 which is a DNA molecule.

3. The **nucleic acid** molecule as claimed in claim 1 which is an RNA molecule.

4. A vector comprising a **nucleic acid** molecule as claimed in claim 1.

5. The vector as claimed in claim 4 comprising one or more regulatory elements which ensure the transcription of said **nucleic acid** molecules and/or the synthesis of a translatable RNA in a pro- and/or eukaryotic cell.

6. The vector as claimed in claim 4, wherein said **nucleic acid** molecule is linked in sense orientation to regulatory elements which ensure the transcription and synthesis of a translatable RNA in pro- and/or eukaryotic cells, or wherein said **nucleic acid** molecule is linked in anti-sense orientation to regulatory elements which ensure the transcription and synthesis of a non-translatable RNA in pro- and/or eukaryotic cells.

7. A host cell which is transformed with a **nucleic acid** molecule as claimed in claim 1 or a vector as claimed in claim 4, or a cell which is derived from the host cell and which comprises the vector of claim 4.

8. The host cell as claimed in claim 7 which is a plant cell.

9. A method for producing a protein encoded by the **nucleic acid** molecule of claim 1, in which a host cell as claimed in claim 7 is cultured under conditions which permit the synthesis of the protein, and the protein is isolated from the cultured cells and/or the culture medium.

10. The plant cell of claim 8, wherein said **nucleic acid** molecule which encodes a protein with the bioactivity of a starch synthase is under the control of regulatory elements which permit the transcription of a translatable mRNA in plant cells.

11. The plant cell of claim 8, wherein the activity of a protein encoded by the **nucleic acid** molecule of claim 1 is increased in this plant cell compared with corresponding, non-genetically-modified plant cells from wild-type plants.

12. A plant comprising plant cells as claimed in claim 8.

13. The plant as claimed in claim 12 which is a crop plant.

14. The plant as claimed in claim 12 which is a starch-storing plant.

15. The plant as claimed in claim 12 which is a maize plant.

16. A method for generating a transgenic plant cell, wherein a plant cell is subjected to genetic modification by introducing a *nucleic acid* molecule as claimed in claim 1 and/or a vector as claimed in claim 4.

17. A method for generating a transgenic plant, wherein

(a) a plant cell is subjected to genetic modification by introducing a *nucleic acid* molecule as claimed in claim 1 and/or a vector as claimed in claim 4; and

(b) a plant is regenerated from this cell; and, if appropriate,

(c) more plants are generated from the plant of (b).

18. Propagation material of a plant comprising plant cells as claimed in claim 8.

19. A method for producing a modified starch obtained from the host cell of claim 8, from the plant of claim 12, or from the propagation material of 18, comprising the step of extracting the starch from a plant cell as claimed in claim 8, from a plant as claimed in claim 12 and/or from propagation material as claimed in claim 18.

United States Patent

6,586,215

Yaver, et al.

July 1, 2003

Polypeptides having peroxidase activity and nucleic acids encoding same

Abstract

The present invention relates to isolated polypeptides having peroxidase activity and isolated nucleic acid sequences encoding the polypeptides. The invention also relates to nucleic acid constructs, vectors, and host cells comprising the nucleic acid sequences as well as methods for producing and using the polypeptides.

Inventors: Yaver; Debbie (Davis, CA); McArdle; Barbara (Davis, CA)

Assignee: Novozymes Biotech, Inc. (Davis, CA)

Appl. No.: 885329

Filed: June 19, 2001

Current U.S. Class: 435/192; 435/6; 435/320.1; 435/325; 435/252.3;
536/23.1; 536/23.2

Intern'l Class: C12N 009/08; C12N 015/00; C12N 005/00; C12Q
001/68; C07H 021/04

Field of Search: 435/192,6,252.3,320.1 536/23.2,23.1

References Cited [Referenced By]

Other References

Mester et al., 1998, Journal of Biochemistry 273: 15412-15417.

Primary Examiner: Monshipouri; M.

Attorney, Agent or Firm: Stames; Robert L.

Parent Case Text

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of U.S. application Ser. No. 09/596,824 filed Jun. 19, 2000 now U.S. Pat. No. 6,372,464 issued Apr. 16, 2002, which application is fully incorporated herein by reference.

Claims

What is claimed is:

1. An isolated *nucleic acid* sequence encoding a polypeptide having peroxidase activity, selected from the group consisting of:

(a) a *nucleic acid* sequence encoding a polypeptide having an amino acid sequence which has at least 75% *identity* with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6, or at least 85% *identity* with amino acids 22 to 385 of SEQ ID NO:4;

(b) a *nucleic acid* sequence encoding a polypeptide having an amino acid sequence which has at least 75% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5, or at least 85% homology with nucleotides 2008 to 3462 of SEQ ID NO:3;

(c) a *nucleic acid* sequence which hybridizes under high stringency conditions with (i) nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2848 to 4247 of SEQ ID NO:5, (ii) the cDNA sequence contained in nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2848 to 4247 of SEQ ID NO:5, or (iii) a complementary strand of (i) or (ii); and

(d) a fragment of (a), (b), or (c), which encodes a polypeptide having peroxidase activity.

2. The *nucleic acid* sequence of claim 1, which encodes a polypeptide having an amino acid sequence which has at least 75% *identity* with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6.

3. The *nucleic acid* sequence of claim 2, which encodes a polypeptide having an amino acid sequence which has at least 80% *identity* with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6.

4. The *nucleic acid* sequence of claim 3, which encodes a polypeptide of having an amino acid sequence which has at least 85% *identity* with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6.

5. The *nucleic acid* sequence of claim 4, which encodes a polypeptide having an amino acid sequence which has at least 90% *identity* with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6.

6. The *nucleic acid* sequence of claim 5, which encodes a polypeptide having an amino

acid sequence which has at least 95% *identity* with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6 .

7. The ***nucleic acid*** sequence of claim 1, which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6 .

8. The ***nucleic acid*** sequence of claim 1, which encodes a polypeptide consisting of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6, or a fragment thereof having peroxidase activity.

9. The ***nucleic acid*** sequence of claim 1, which encodes a polypeptide consisting of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6.

10. The ***nucleic acid*** sequence of claim 1, which encodes a polypeptide which consists of amino acids 22 to 370 of SEQ ID NO:2, amino acids 22 to 365 of SEQ ID NO:4, or amino acids 19 to 362 of SEQ ID NO:6.

11. The ***nucleic acid*** sequence of claim 1, which has at least 75% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.

12. The ***nucleic acid*** sequence of claim 11, which has at least 80% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.

13. The ***nucleic acid*** sequence of claim 12, which has at least 85% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.

14. The ***nucleic acid*** sequence of claim 13, which has at least 90% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.

15. The ***nucleic acid*** sequence of claim 14, which has at least 95% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.

16. The ***nucleic acid*** sequence of claim 1, which has the ***nucleic acid*** sequence of SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:5.

17. The ***nucleic acid*** sequence of claim 1, which has the ***nucleic acid*** sequence of nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2848 to 4247 of SEQ ID NO:5.

18. The ***nucleic acid*** sequence of claim 1, which hybridizes under high stringency conditions with (i) nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2848 to 4247 of SEQ ID NO:5, (ii) the cDNA sequence contained in nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2845 to 4247 of SEQ ID NO:5, or (iii) a complementary strand of (i) or (ii).

19. The *nucleic acid* sequence of claim 1, which is contained in plasmid pBM37-7 which is contained in E. coli NRRL B-30280, plasmid pBM38-1 which is contained in E. coli NRRL B-30281, or plasmid pBM39-1 which is contained in E. coli NRRL B-30282.

20. A *nucleic acid* construct comprising the *nucleic acid* sequence of claim 1, operably linked to one or more control sequences which direct the production of the polypeptide in a suitable expression host.

21. A recombinant expression vector comprising the *nucleic acid* construct of claim 20, a promoter, and transcriptional and translational stop signals.

22. A recombinant host cell comprising the *nucleic acid* construct of claim 20.

23. A method for producing a polypeptide having peroxidase activity comprising

(a) cultivating the host cell of claim 22, under conditions suitable for production of the polypeptide; and

(b) recovering the polypeptide.

United States Patent

6,586,179

Jegla, et al.

July 1, 2003

Human Eag2

Abstract

The invention provides isolated nucleic acid and amino acid sequences of Eag2, antibodies to Eag2, methods of detecting Eag2, and methods of screening for modulators of Eag2 potassium channels using biologically active Eag2. The invention further provides, in a computer system, a method of screening for mutations of human Eag2 genes as well as a method for identifying a three-dimensional structure of Eag2 polypeptide monomers.

Inventors: **Jegla; Timothy J.** (Durham, NC); **Liu; Yi** (Cary, NC)

Assignee: **ICAgen, Incorporated** (Durham, NC)

Appl. No.: **614480**

Filed: **July 10, 2000**

Current U.S. Class: 435/6; 536/23.1; 435/69.1; 435/325; 435/320.1;
435/252.3; 530/350

Intern'l Class: C12Q 001/68; C07H 017/00; C12P 021/06; C07K
014/00

Field of Search: 536/23.1 435/7.1,325,320.1,252.3 530/350 436/6

References Cited [Referenced By]

Other References

Ludwig et al. 1994; EMBO J. 13: 4451-4458.*

Volario et al. 1998; DNA Seq. 9(5-6):307-315.*

Bruggemann, Andrea et al., "Ether-a-go-go encodes a voltage-gated channel permeable to K^{sup.+} and Ca^{sup.2+} and modulated by cAMP," Nature 365:445-448 (Sep. 1993).

Frings, Stephan et al., "Characterization of Ether-a-go-go Channels Present in Photoreceptors Reveals Similarity to I_{sub.KX}, a K^{sup.+} Current in Rod Inner Segments," J. Gen. Physiol. 111:583-599 (Apr. 1998).

Occhiodoro, Teresa et al., "Cloning of a human ether-a-go-go potassium channel expressed in myoblasts at the onset of fusion," Federation of European Biochemical Societies 434:177-182 (1998).

Robertson, G. A. et al., "Potassium Currents Expressed from Drosophila and Mouse eag cDNAs in Xenopus Oocytes." Neuropharmacology 35(7):841-850

(1996).

Shi, Wenmei et al., "Cloning of a mammalian elk potassium channel gene and EAG mRNA distribution in rat sympathetic ganglia," Journal of Physiology 511(3):675-682 (1998).

Warmke, Jeffrey et al., "A Distinct Potassium Channel Polypeptide Encoded by the Drosophila eag Locus," Science 252:1560-1562 (1991).

Saganich et al., "Cloning of Components of a Novel Subthreshold-Activating K_{sup}+ Channel With a Unique Pattern of Expression in the Cerebral Cortex," J. Neuroscience, vol. 19, No. 24, pp. 10789-10802 (1999).

Primary Examiner: Carlson; Karen Cochrane

Attorney, Agent or Firm: Townsend and Townsend and Crew LLP

Parent Case Text

CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims priority to U.S. Ser. No. 60/143,467, filed Jul. 13, 1999, herein incorporated by reference in its entirety.

Claims

What is claimed is:

1. An isolated ***nucleic acid*** encoding a polypeptide comprising an alpha subunit of a potassium channel, wherein the subunit:

(i) forms, with at least one additional Eag family alpha subunit, a potassium channel having the characteristic of voltage sensitivity; and wherein said ***nucleic acid*** specifically hybridizes under stringent conditions to SEQ ID NO:1, wherein the hybridization reaction is incubated at 42.degree. C. in a solution comprising 50% formamide, 5.times.SSC, and 1% SDS or at 65.degree. C. in a solution comprising 5.times.SSC and 1% SDS, with a wash in 0.2.times.SSC and 0.1% SDS at 65.degree. C.

2. An isolated ***nucleic acid*** encoding a polypeptide comprising an alpha subunit of a potassium channel, wherein the subunit:

(i) forms, with at least one additional Eag family alpha subunit, a potassium channel having the characteristic of voltage sensitivity; and

(ii) comprises an amino acid sequence that has greater than 85% amino acid ***identity*** to the amino acid sequence of SEQ ID NO:2.

3. The isolated *nucleic acid* of claim 1, wherein the polypeptide specifically binds to polyclonal antibodies generated against SEQ ID NO:2.
4. The isolated *nucleic acid* of claim 1, wherein the *nucleic acid* encodes human Eag2.
5. The isolated acid of claim 1, wherein the *nucleic acid* encodes an amino acid sequence of SEQ ID NO:2.
6. The isolated *nucleic acid* sequence of claim 1, wherein the *nucleic acid* has a nucleotide sequence of SEQ ID NO:1.

7. The isolated *nucleic acid* of claim 1, wherein the *nucleic acid* is amplified by primers that selectively hybridize under stringent hybridization conditions to the same sequence as primers selected from the group consisting of:

ATGCCGGGGGGCAAGAGAGGGCTG (SEQ ID NO:3);

CTGACCCTAAGCTCATAAGGATGAAC (SEQ ID NO:4);

CCACCTCATCATCCTGGATGACTTCC (SEQ ID NO:5);

TTAAAAGTGGATTTCATCTTTGTCAGATTCAGG (SEQ ID NO :6);

GGGGACCTCATTTACCATGCTGGAG (SEQ ID NO:7);

GATTCCCTCATCCACATTTTCAAAGGC (SEQ ID NO:8);

and wherein the hybridization reaction is incubated at 42.degree. C. in a solution comprising 50% formamide, 5.times.SSC, and 1% SDS or at 65.degree. C. in a solution comprising 5.times.SSC and 1% SDS, with a wash in 0.2.times.SSC and 0.1% SDS at 65.degree. C.

8. The isolated *nucleic acid* of claim 1, wherein the polypeptide monomer comprises an alpha subunit of a homomeric channel.
9. The isolated *nucleic acid* of claim 1, wherein the polypeptide monomer comprises an alpha subunit of a heteromeric channel.
10. An expression vector comprising the *nucleic acid* of claim 1.
11. A host cell transfected with the vector of claim 10.
12. A method of detecting a *nucleic acid*, the method comprising contacting a sample comprising a first *nucleic acid* with an isolated second nucleic acid of claim 1 and

USPN 6,586,179

detecting hybridization of the second *nucleic acid* to the first *nucleic acid*, thereby detecting the first *nucleic acid*.

United States Patent

6,583,337

Allen , et al.

June 24, 2003

Plant glucose-6-phosphate translocator

Abstract

This invention relates to an isolated nucleic acid fragment encoding a glucose-6-phosphate/phosphate translocator. The invention also relates to the construction of a chimeric gene encoding all or a portion of the glucose-6-phosphate/phosphate translocator, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the glucose-6-phosphate/phosphate translocator in a transformed host cell.

Inventors: **Allen; Stephen M.** (Wilmington, DE); **Rafalski; J. Antoni** (Wilmington, DE)

Assignee: **E. I. du Pont de Nemours and Company** (Wilmington, DE)

Appl. No.: **436521**

Filed: **November 9, 1999**

Current U.S. Class: 800/278; 435/6; 435/69.1; 435/71.1; 435/183;
435/410; 435/419; 435/418; 435/252.3; 435/320.1;
530/350; 530/370; 536/23.1; 536/23.2; 536/23.6;
536/24.1; 536/24.3; 536/24.5

Intern'l Class: A01H 003/00; C07H 021/04; C07K 014/415; C12N
005/14; C12N 009/00

Field of Search: 435/6,69.1,71.1,183,410,419,418,252.3,320.1
530/370,350 536/23.1,23.2,23.6,24.1,24.3,24.5

References Cited [Referenced By]

U.S. Patent Documents

<u>5945509</u>	Aug., 1999	Heinemann et al.
----------------	------------	------------------

Foreign Patent Documents

WO 95/16913	Jun., 1995	WO.
-------------	------------	-----

Other References

Lazar et al. Molecular and Cellular Biology, vol. 8, No. 3, p. 1247-1252, Mar. 1988.*

Burgess et al. The Journal of Cell Biology, vol. 111 p. 2129-2138, 1990.*
Brown et al. Science, vol. 282, p. 131-133, Nov. 1998.*
Bork. Genome Research vol. 10, p. 398-400, 2000.*
Kammerer, B. et al. (1998) The Plant Cell 10:105-117.
Denyer et al. (1996) Plant Physiol. 112:779-785.
Thorbjornsen et al. (1996) Plant J. 10:243-250.
NCBI General Identifier No. 2997591.
NCBI General Identifier No. 2997589.

Primary Examiner: Bui; Phuong T.

Parent Case Text

This application claims priority benefit to U.S. Provisional Application No. 60/107,910 filed Nov. 10, 1998, now abandoned.

Claims

What is claimed is:

1. An isolated ***polynucleotide*** comprising:

(a) a nucleotide sequence encoding a polypeptide having glucose-6-phosphate/phosphate translocator activity, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:4 have at least 86% sequence ***identity*** based on the Clustal alignment method, or

(b) the complement of the nucleotide sequence, wherein the complement and the nucleotide sequence contain the same number of nucleotides and are 100% complementary.

2. The ***polynucleotide*** of claim 1, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:4 have at least 90% sequence ***identity*** based on the Clustal alignment method.

3. The ***polynucleotide*** of claim 1, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:4 have at least 95% sequence ***identity*** based on the Clustal alignment method.

4. The ***polynucleotide*** of claim 1, wherein the nucleotide sequence comprises the nucleotide sequence of SEQ ID NO:3.

5. The *polynucleotide* of claim 1, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:4.
6. A recombinant DNA construct comprising the *polynucleotide* of claim 1 operably linked to a regulatory sequence.
7. A method for transforming a cell comprising transforming a cell with the *polynucleotide* of claim 1.
8. A cell comprising the recombinant DNA construct of claim 6.
9. A method for producing a plant comprising transforming a plant cell with the *polynucleotide* of claim 1 and regenerating a plant from the transformed plant cell.
10. A plant comprising the recombinant DNA construct of claim 1.
11. A seed comprising the recombinant DNA construct of claim 1.
12. A vector comprising the *polynucleotide* of claim 1.

United States Patent

6,582,950

Smith, et al.

June 24, 2003

C3 binding polypeptide of Streptococcus agalactiae group b Streptococcus

Abstract

This invention relates to the identification of a human complement C3 binding polypeptide and the nucleic acid which encodes the polypeptide from Streptococcus agalactiae. The polypeptide binds C3 and may be implicated in S. agalactiae adhesion and/or virulence. The polypeptide is conserved in mass in a variety of streptococcal isolates and is recognized by antibodies produced by humans exposed to or colonized with Group B Streptococcus.

Inventors: **Smith; Beverly L.** (Minneapolis, MN); **Ferrieri; Patricia** (Minneapolis, MN)

Assignee: **Regents of the University of Minnesota** (Minneapolis, MN)

Appl. No.: **610199**

Filed: **July 1, 2000**

Current U.S. Class: 435/252.3; 435/320.1; 435/325; 536/23.7

Intern'l Class: C12N 001/20; C12N 015/00; C12N 005/00; C07H 021/04

Field of Search: 424/190.1 435/69.3,252.33,253.4,252.3,320.1,325 536/23.7

References Cited [Referenced By]

U.S. Patent Documents

5302527 Apr., 1994 Birkett et al.

5648241 Jul., 1997 Michel et al.

Foreign Patent Documents

WO 98/21337 May., 1998 WO.

Other References

Teti et al., "Adherence of Group B Streptococci to Adult and Neonatal Epithelial Cells Mediated by Lipoteichoic Acid," Infection and Immunity, 1987 Dec.; vol. 55(12):3057-3064.

"A Group B Strep Vaccine!?" Medical Advisory Board, Group B Strep

3064 (1987).

Wastfelt et al., "Identification of a Family of Streptococcal Surface Proteins with Extremely Repetitive Structure," *The Journal of Biological Chemistry*, 271(31):18892-18897 (1996).

Wattiaux, "Topic # 5, Mastitis: The Disease and its Transmission," *Babcock Dairy Essentials, Topic Summaries from: Lactating and Milking, Technical Dairy Guide*, [online]. The Babcock Institute, International Dairy Research and Development, University of Wisconsin, copyright 1996 [retrieved Aug. 25, 1999]. Retrieved from the Internet <URL: <http://babcock.cals.wisc.edu/>>, 4 pages.

Wessels et al., "Immunogenicity in Animals of a Polysaccharide-Protein Conjugate Vaccine against Type III Group B Streptococcus," *The Journal of Clinical Investigation*, 86(5):1428-1433 (1990).

Wessels et al., "Immunogenicity and Protective Activity in Animals of a Type V Group B Streptococcal Polysaccharide-Tetanus Toxoid Conjugate Vaccine," *The Journal of Infectious Diseases*, 171(4):879-884 (1995).

Wessels et al., "Structural Properties of Group B Streptococcal Type III Polysaccharide Conjugate Vaccines That Influence Immunogenicity and Efficacy," *Infection and Immunity*, 66(5):2186-2192 (1998).

Wilkinson, "Type-Specific Antigens of Group B Type Ic Streptococci," *Infection and Immunity*, 4(5):596-604 (1971).

Wilkinson, "Comparison of Streptococcal R Antigens," *Applied Microbiology*, 24(4):669-670 (1972).

Primary Examiner: Navarro; Mark

Attorney, Agent or Firm: Mueting Raasch & Gebhardt

Parent Case Text

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. .sectn.119(e) of U.S. Provisional Patent Application No. 60/157,550, filed on Oct. 4, 1999, and U.S. Provisional Patent Application No. 60/173,766, filed on Dec. 30, 1999, both of which are hereby incorporated by reference.

Claims

What is claimed is:

1. An isolated *nucleic acid* fragment that hybridizes to at least a portion of at least one of the *nucleic acid* fragments represented by SEQ ID NO:6 or SEQ ID NO:4 or their

complementary strands under hybridization conditions of prehybridization for 1 hour at 62.degree. C. in hybridization solution (5.times.SSC (1.times.SSC is 0.15 M NaCL, 0.015 M sodium citrate), 0.02% sodium dodecyl sulfate (SDS), 0.1% N-lauroylsarcosine, 1% Blocking Reagent) followed by two stringency washes with 2.times.SSC, 0.1% SDS for 5 minutes at room temperature and once with 0.5.times.SSC, 0.1% SDS for 15 minutes at 62.degree. C., said isolated **nucleic acid** fragment encodes a polypeptide that binds human complement C3 protein.

2. The **nucleic acid** fragment of claim 1 isolated from *S. agalactiae*.
3. The **nucleic acid** fragment of claim 1 which encodes a polypeptide represented by SEQ ID NO:5.
4. The **nucleic acid** fragment of claim 1 in a **nucleic acid** vector.
5. The **nucleic acid** fragment of claim 4 wherein the **nucleic acid** vector is an expression vector capable of producing a polypeptide.
6. An isolated **nucleic acid** having at least 50% **nucleic acid identity** to the **nucleic acid** fragments represented by SEQ ID NO:6 or SEQ ID NO:4, and which hybridizes under hybridization conditions of prehybridization for 1 hour at 62.degree. C. in hybridization solution (5.times.SSC (1.times.SSC is 0.15 M NaCL, 0.015 M sodium citrate), 0.02% sodium dodecyl sulfate (SDS), 0.1% N-lauroylsarcosine, 1% Blocking Reagent) followed by two stringency washes with 2.times.SSC, 0.1% SDS for 5 minutes at room temperature and once with 0.5.times.SSC, 0.1% SDS for 15 minutes at 62.degree. C., to at least a portion of at least one of the **nucleic acid** fragments represented by SEQ ID NO:6 or SEQ ID NO:4 or their complementary strands, said **nucleic acid** encoding a polypeptide that binds human complement C3 protein.
7. An isolated **polynucleotide** encoding a polypeptide comprising the amino acids represented by SEQ ID NO:5.
8. The **polynucleotide** of claim 7 wherein the polypeptide binds human complement C3.
9. An isolated host cell comprising a **nucleic acid** fragment of claim 1.
10. The cell of claim 9 wherein the cell is a bacterium or a eukaryotic cell.
11. An isolated **nucleic acid** fragment comprising SEQ ID NO:6 or SEQ ID NO:4 or their complementary strands.
12. An isolated RNA transcribed from a double-stranded **nucleic acid** comprising a **nucleic acid** fragment of claim 2.
13. An isolated **nucleic acid** fragment encoding a polypeptide having at least 50% amino acid **identity** to SEQ ID NO:5, said polypeptide binds to human complement C3 protein.

14. An isolated *nucleic acid* fragment encoding a polypeptide having at least 60% amino acid *identity* to SEQ ID NO:5, said polypeptide binds to human complement C3 protein.
15. An isolated *nucleic acid* fragment encoding a polypeptide having at least 70% amino acid *identity* to SEQ ID NO:5, said polypeptide binds to human complement C3 protein.
16. An isolated *nucleic acid* fragment encoding a polypeptide having at least 80% amino acid *identity* to SEQ ID NO:5, said polypeptide binds to human complement C3 protein.
17. An isolated *nucleic acid* fragment consisting essentially of at least 30 nucleotides of SEQ ID NO:4, wherein said *nucleic acid* fragment encodes a polypeptide that binds to human complement C3 protein.

United States Patent

6,541,684

Bowen, et al.

April 1, 2003

Nucleotide sequences encoding maize RAD51

Abstract

Nucleic acid sequences encoding two RAD51 recombinases active in maize plants are provided. cDNA sequences including the ZmRAD51 coding sequences and unique 3'-untranslated regions which are useful as RFLP probes, are also provided. The production of plasmids containing a nucleic acid sequence encoding a ZmRAD51 fusion protein, as well as the use of the plasmids to introduce the ZmRAD51 coding sequence into a host cell, such as maize cell, are also disclosed.

Inventors: **Bowen; Benjamin A.** (Hayward, CA); **Chamberlin; Mark A.** (Windsor Heights, IA); **Drummond; Bruce J.** (Des Moines, IA); **McElver; John A.** (Durham, NC); **Rothstein; Rodney J.** (Maplewood, NJ)

Assignee: **Trustees of Columbia University in the City of New York** (New York, NY); **Pioneer Hi-Bred International, Inc.** (Johnston, IA)

Appl. No.: **246963**

Filed: **February 9, 1999**

Current U.S. Class: 800/320.1; 435/69.1; 435/196; 536/23.1; 536/23.5; 536/24.1

Intern'l Class: A01H 005/00

Field of Search: 536/23.1,23.5,24.1 435/410,468,196,69.1
800/298,320.1

References Cited [Referenced By]

Foreign Patent Documents

WO 93/22443	Nov., 1993	WO.
WO 97/41228	Nov., 1997	WO.

Other References

Natalie Teager et al, Isolation and characterization of rad51 orthologs from *Coprinus cinereus* and *Lycopersicon esculentum*, and phylogenetic analysis of eukaryotic recA homologs, *Curr Genet* 1997, 31: 144-157.*
Kowalczykowski, et al. 1994. Homologous Pairing and DNA Strand-Exchange Proteins. *Annu Rev. Biochem.* 1994. 63.99 -1043.

Sung, 1994. Catalysis of ATP-Dependent Homologous DNA Pairing and Strand Exchange by Yeast RAD51 Protein. Science, vol. 265, 1241-1243.

Stassen, et al. Isolation and characterization of rad51 orthologs from Coprinus cinereus and Lycopersicon esculentum, and phylogenetic analysis of eukaryotic recA homologs. Curr Genet (1997) 31: 144-157.

Doutriaux, et al. Isolation and characterisation of the RAD51 and DMC1 homologs from Arabidopsis thaliana. Mol Gen. Genet (1998) 257: 283-291.

Kanaar and Hoeijmakers, 1998. From competition to collaboration. Nature, vol. 391, 335-338.

Benson, et al. 1998. Synergistic actions of Rad51 and Rad52 in recombination and DNA repair. Nature, vol. 391, 401-404.

Shinohara and Ogawa, 1998. Stimulation by Rad52 of yeast Rad51-mediated recombination. Nature, 391: 404-407.

New, et al., 1998. Rad 52 protein stimulates DNA strand exchange by Rad51 and replication protein A. Nature, 391: 407-410.

Smith, K.N., et al. 1996. Untitled. Embl. Sequence Data Library, XP002105502, Accession No. U43528.

Primary Examiner: Ketter; James

Attorney, Agent or Firm: Foley & Lardner

Parent Case Text

CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 60/074,745, filed Feb. 13, 1998 and is herein incorporated by reference.

Claims

What is claimed is:

1. An isolated polynucleotide comprising a member selected from the group consisting of:
 - a) a polynucleotide encoding a polypeptide selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO: 7;
 - b) a polynucleotide having at least 90% identity to a polynucleotide of (a);
 - c) a polynucleotide which will hybridize under *stringent hybridization* conditions to said polynucleotide of (a) or (b); and

d) a polynucleotide comprising at least 30 contiguous nucleotides from a polynucleotide of (a), (b) or (c);

wherein the polynucleotide of (a), (b) or (c) encodes a polypeptide with recombinase activity.

2. The isolated polynucleotide of claim 1, wherein said polynucleotide has a sequence selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO: 6.

3. An expression cassette comprising a polynucleotide of claim 1 operably linked to a promoter.

4. The host cell transfected with an expression cassette of claim 3.

5. The host cell of claim 4, wherein said host cell is a bacterial cell.

6. The host cell of claim 4, wherein said host cell is a sorghum or maize cell.

7. A method of making maize recombinase comprising the steps of:

a) transforming or transfecting a host cell with the expression cassette of claim 3; and

b) purifying the recombinase from the host cell.

8. The method of claim 7, wherein the host cell is selected from the group consisting of a bacterial cell, a plant cell, a mammalian cell and a yeast cell.

9. A method of modulating ZmRAD 51 activity in a plant, comprising:

(a) introducing into a plant cell an expression cassette comprising an isolated polynucleotide of claim 1 operatively linked to a promoter;

(b) culturing the plant cell under plant cell growing conditions;

(c) regenerating a plant which possesses the transformed genotype, and

(d) inducing expression of said polynucleotide for a time sufficient to modulate ZmRAD51 activity in said plant.

10. A transgenic plant cell comprising an isolated polynucleotide of claim 1.

11. A transgenic plant comprising an isolated polynucleotide of claim 1.

12. A transgenic seed from the transgenic plant of claim 11.

USPN 6,541,684

13. Primer pairs for isolating at least a part of a Zea mays recombinase gene, selected from the group consisting of SEQ ID NOS: 12 and 13, SEQ ID NOS: 14 and 19, SEQ IDS NOS: 14 and 20, and SEQ ID NOS: 14 and 15, or complements thereof.

14. An RFLP probe for a maize recombinase gene comprising at least 30 nucleotides residues of SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, or SEQ ID NO: 11.